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14. ABSTRACT

The Cooperative International Neuromuscular Research Group (CINRG) is a consortium of medical and scientific investigators and centers focused on improving quality of life of neuromuscular disease (NMD) patients by cooperatively conducting clinical research studies in NMD. The CINRG Coordinating Center (CC) is an infrastructure to support the network in conducting study protocols, standardizing clinical trial methodology, data collection and quality assurance, and performing data analyses. In this award period, we have continued to make progress on the revamping of the CINRG quantitative measurement system (CQMS); we have completed sixteen CINRG site visits for hands-on training of site physical therapists as well as monitoring of study procedures and data collection, four new grant applications were submitted, two new protocols were developed, and one manuscript was published. Two additional manuscripts were submitted and several others are in draft form. This infrastructure support yields better overall results in research for improving care in DMD in the largest network of institutions for this disease worldwide.

15. SUBJECT TERMS
Duchenne Muscular Dystrophy, CINRG, CQMS, Coordinating Center, Electronic Data Capture

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1. Introduction

Duchenne muscular dystrophy (DMD) is a rare disease occurring in 1 of 3,500 live born males worldwide. The Cooperative International Neuromuscular Research Group (CINRG) is a consortium of medical and scientific investigators from academic and research centers sharing a common goal of improving the quality of life of neuromuscular disease patients by cooperative planning, implementation, analysis and reporting of controlled clinical studies and of other research for neuromuscular disease. In order to support CINRG in its efforts to perform the highest quality of research, a Coordinating Center (CC) is required to coordinate efforts and protocols, standardize methods of clinical trial treatment administration and assessments, as well as data collection and quality assurance, and analyses of data. The goal of this project is to provide the CINRG clinical research network with an infrastructure for operational support to conduct its studies, database and data management support for collection of data from CINRG studies, specific support in training clinical evaluators (CEs) for muscle strength and biostatistical support for study design, assessment of feasibility and analysis of study results, as well as supporting new grant submissions. The CINRG CC will provide a centralized administrative and technical infrastructure to meet the complex needs of the program that is supportive of CINRG's scientific agenda.

2. Body

2.1 Revamp of the CINRG Quantitative Measurement System (CQMS)

In the previous year (Year 2) of this grant, a contract was signed between CINRG and Near Infinity Corporation (NIC) to design a software system that is compatible with the current CINRG quantitative measurement system (CQMS) hardware and to include encrypted data transmission to CINRG's web-based data entry system, which is in OpenClinica. The contract also included updating the audio-visual game component which encourages participants to exert maximal effort in these evaluations. Milestone 1 of this contract was completed in Year 2. In Year 3, we continued to work with NIC on the development of the new CQMS software (CQMS3) for Milestones 2 – 7. Through these milestones we will have the ability to manage and design clinical assessments for new study designs and new assessments, and create import/export of data between OpenClinica and CQMS3 so that all study data resides in a single database. The information read into CQMS3 from OpenClinica will decrease repeated data entry of basic study and demographic data as well as direct skip (branching) patterns related to assessments required by the specific studies in CQMS3. This functionality exists only in a limited way in the current CQMS implementation.

Discoveries in both OpenClinica's functionality and performance related to the information exchange with CQMS3 software required additional developments in the software critical to the functionality of the CQMS software. These modifications contributed to delays in the original timeline and required an amendment to extend the contract. CINRG and NIC are currently in the process of finalizing an additional amendment that includes development of coding for skip patterns in OpenClinica and FTP raw data storage on a secure server. These enhancements are expected to greatly improve data quality, integration and the user experience with the new software.

Currently, successful alpha testing has occurred with the CQMS3 software system with the CC. The system is functional in that a study design can be done in OpenClinica, which will define which parameters need to be collected in the CQMS3 system. Measurements of muscle strength are received by the CQMS3 system, correctly attributable to the specific patient, visit, date, muscle, side (right or left), and replicate. The data uploads correctly to OpenClinica, via the computing interface between these two software products. The gaming component responds differently according to strength to encourage patients of all strength abilities, from as strong as normal adults and as weak as DMD patients who lost ambulation, but can still do a grip test. The graphics are visually attractive and will add motivation and encouragement to exert the most by subjects. With a few more minor corrections, the software is at the point of being sent out to 5 other CINRG sites for beta testing.

2.2 Training of new clinical evaluators

Training of new CEs in Year 3 focused on hands on instruction for standardized test assessments in muscle strength, anthropometric measurements, functional and timed tests that are part of the CINRG clinical outcomes toolbox. Reliability testing was performed with all newly trained clinical evaluators to ensure reproducibility of testing.

The Clinical Evaluations Manager (CEM) visited 8 CINRG sites. A total of 11 new CEs were certified as CINRG CEs and an additional 10 existing CEs were recertified. A second CE was also certified as a CEM to perform CE certifications, reliability and outcome management at the CINRG CC. Below is a detailed review of the completed site visits and certifications:

- Apollo Hospitals in Chennai, India: From January 25th to 28th, 2012, the CEM trained 2 new CEs and recertified 1 CE. As a reminder, this site changed location, from one hospital to another, and was approved by CINRG's Executive Committee based on the new hospital's facility information and being directed by one of CINRG's more seasoned investigators. This was the first monitoring visit in the new location. The CEM assessed equipment, testing space and logistics to ensure the site meets the minimal testing requirements for CINRG. During this visit, the CEM also reviewed the site personnel regulatory binders and collected appropriate updated personnel documents and addressed missing items.
- National Center for Neurology and Psychiatry in Tokyo, Japan: From February 6th to 8th, 2012, the CEM certified 3 new CEs. Equipment, supplies and space were assessed and met the minimal testing requirements to perform CINRG clinical evaluations.
- Kobe University in Kobe, Japan: On February 9th, 2012, the CEM performed a site assessment visit. This site was approved to join the CINRG network in Year 2. During this visit, the CEM identified logistical hurdles such as institution affiliation of site personnel and worked with the site on identifying appropriate testing areas for the installation of the CQMS equipment.
- Centro Clinico NEMO in Milan, Italy: From February 20th to 22nd, 2012, the CEM certified 2 new CEs. Additionally, 2 CINRG CEs from Sweden traveled to Milan and were recertified as CINRG CEs. Equipment, supplies and space were assessed and the CEM required re-installation of the CQMS bars for safety purposes.
- University of Pittsburgh in Pittsburgh, PA: From April 13th to 14th, 2012, the CEM certified a second CEM to perform future CE training visits and recertified 2 existing CEs. Equipment, supplies and space were assessed and met the minimal test requirements to perform CINRG evaluations.
- University of Puerto Rico in San Juan, Puerto Rico: From June 26th to 28th, 2012, the CEM certified 1 new CE and recertified 1 existing CE. Equipment, supplies and space were assessed and met the minimal test requirements to perform CINRG evaluations.
- Texas Children's Hospital in Houston, TX: From August 1st to 3rd, 2012, the CEM certified 2 new CEs and recertified 1 existing CE. The site intends to move their current CQMS equipment from one testing area to another and the CEM also assessed the new space. The existing and future locations appear to be sufficient for standardized CINRG

evaluations. Equipment and supplies were also assessed and met minimal requirements for test performance.

- University of Minneapolis in Minneapolis, MN: From August 13th to 15th, 2012, the CEM certified 1 new CE and recertified 1 existing CE. Several equipment pieces were found to be malfunctioning and replacement parts were ordered. Equipment issues are anticipated to be resolved by the end of October 2012.

2.3 Updates on Protocols Related to Duchenne Muscular Dystrophy Research Supported by the CINRG Coordinating Center

In this section we have outlined the progress of each CINRG project that relates to DMD research made in Year 3. The first four projects (see Sections **2.3.1, 2.3.2, 2.3.3, and 2.3.4**) represent updates on closed studies. The last four projects (see Sections: **2.3.5, 2.3.6, 2.3.7, and 2.3.8**) represent active and new studies.

2.3.1 National Initiative for Families with Duchenne (NIFD)

A. Overview

The purpose of this survey was to collect information about families of people with DMD all over the USA. The survey asked for information about the impact of DMD on the family, the needs of the family for health services, the use of those health and school support services, the overall wellness of people with DMD and attitudes toward newborn screening for DMD. A total of 237 families participated in this study. Participants were enrolled either through the CINRG DMD Natural History Study (discussed in section **2.3.6**) or directly through the NIFD study completed via a web-based survey. The data management team has merged the collected study data into one dataset.

B. Project Updates

The data management team has been applying a systematic approach to correcting data errors within the survey, section by section. In Year 3, we have received a request to analyze the final two unpublished sections: Your Child's Health and Medical Care. The data management team is currently working with the researcher to prepare the sections for analysis and then analyze the desired areas of interest for publication.

2.3.2 A double-blinded randomized placebo controlled study of daily Pentoxifylline as a rescue treatment in DMD

A. Overview

In this study, Pentoxifylline was added as a rescue treatment to patients who were receiving steroids (prednisone, prednisolone or deflazacort) for at least 12 months in a stable dose regardless of weight change. A total of 64 participants were enrolled. The database was locked in February, 2008.

B. Manuscript

The manuscript was accepted and published in Neurology (see **Key Accomplishments**).

2.3.3 Comparative Study of Clinical Endpoints in DMD: HHM vs. CQMS protocol

A. Overview

The purpose of this study was to compare the commonly used pediatric strength testing measures: handheld myometry (HHM) and CQMS, with the goal of assessing which of these two methods had a higher intra-rater and inter-rater reliability in measuring muscle strength in children with DMD. The database was locked in May 2011.

B. Manuscript Preparation

In Year 3, further data analyses occurred. Previous mixed effect models showed that in this complex four-period two-rater (8 testing sessions over two days) study there were no significant fatigue effects on results of muscle strength testing. Analysis of variance models were used to calculate intra and inter-rater reliability of the clinical evaluators in each of the two strength testing approaches and in four muscle groups (Lu & Shara, 2007, Gwet, 2008, Shrout and Fleiss, 1979). From an instrumental perspective, strength assessments performed by experienced CEs show comparable measurements for both instruments for knee flexion/extension and elbow flexion/extension although the degree of variability was different by muscle.

2.3.4 Cardiac Outcome Measures in Children with Muscular Dystrophy protocol

A. Overview

This project aimed at developing cardiac outcome measures that could be reliably implemented across a consortium of clinical sites devoted to the study of pharmaceutical treatments for muscular dystrophy. This study was funded as a CTSA supplement through the University of Pittsburgh. Funding for this project ended on June 30, 2011 and the two associated studies (one for echocardiographic measures and one for cardiac magnetic resonance measures) were closed.

B. Data Completion and database Lock

Patient visits ended in Year 2 of this grant. Data included echo and ECG readings by two central readers from two institutions. These readings were done mostly in Year 3 when all the materials were available to the central readers. Other relevant clinical data were also collected in this study. The data and project managers worked to get all cardiac data centrally read by both readers, data entered, queried as appropriate, including detection of outliers, and the database was locked.

2.3.5 Clinical Trial of Coenzyme Q10 and Lisinopril in muscular dystrophies

A. Overview

The objective of this study is to test an angiotensin converting enzyme (ACE) inhibitor, lisinopril, and an anti-oxidant, coenzyme Q10 (CoQ10), to ameliorate the decline in cardiac muscle function that occurs in muscular dystrophies. The study treatment period is 24 months per patient. This project is primarily funded by the Department of Defense (grant W81XWH-04-1-0851). The activities that are related to Year 3 for this award cover work performed on regulatory and data management support.

B. Project Updates

The study team has been actively working on enrollment challenges. They are working on adding clinical study sites and encouraging participant screenings at all Department of Defense-approved clinical study sites. The CINRG CC statisticians have also performed several analyses to determine if the samples size could be adjusted or if the cardiac inclusion criteria could be modified (see section **E. Statistical Analysis** below).

C. CINRG Site Updates and Site Monitoring

In Year 3, one additional CINRG center has begun patient recruitment. The study has been updated periodically on www.clinicaltrials.gov to include newly approved sites. In Year 3 we began implementing a new tracking system, Evolve, for all essential site and project documents (see section **2.4.7**). This project was used as the demonstration study for all training modules completed with the new software.

The table below provides a status update for all CINRG centers involved in this protocol.

CINRG Sites	Local Ethics Preparation	Local Ethics Approved	DoD HRPO Approved	Participant recruitment
University of Pittsburgh, Pittsburgh, PA		X	X	X
Children's National Medical Center, Washington, DC		X	X	X
University of Tennessee, Memphis, TN		X	X	X
Alberta Children's Hospital, Calgary, Canada		X	X	X
Carolinas Medical Center, Charlotte, NC		X	X	X
Children's Memorial Hospital, Chicago, IL		X	X	X
Washington University, St. Louis, MO		X	X	X
National Center of Neurology and Psychiatry, Tokyo, Japan		X	X	X
Hadassah Medical Center, Jerusalem, Israel		X	In review	
Apollo Hospitals, Chennai, India		X	In review	
University of California, Sacramento, CA		X	In draft	
Centro Clinico NEMO, Milan, Italy	X			
Children's Hospital of Westmead, Sydney, Australia	X			
Duke Medical Center, Durham, NC	X			
Kobe University, Kobe, Japan	X			

In Year 3 the project management team visited the following 4 CINRG sites to initiate and monitor this study:

- University of Tennessee in Memphis, TN for a site initiation visit.
- National Center of Neurology and Psychiatry in Tokyo, Japan for a site initiation visit.
- Alberta Children's Hospital in Calgary, Canada for an interim monitoring visit.
- University of Pittsburgh in Pittsburgh, PA for an interim monitoring visit.

D. Data Management

In Year 3, the electronic data capture (EDC) system OpenClinica has been continually maintained. OpenClinica released an updated version in Year 3 with new skip pattern (branching) features that required the data team to upgrade all electronic case report forms for this study. This upgrade required the data management team to hold a training session for all current users to implement the new improved skip pattern features. The data management team also finalized the Conclusion of Study forms and circulated these forms to all active sites. Finally, the data management team also completed OpenClinica training for new CINRG site staff members.

E. Statistical Analysis

In Year 3 the CINRG CC statisticians performed several analyses to assist the study chair and the data and safety monitoring board (DSMB) with possible decisions to modify the protocol to address enrollment challenges. Below is a detailed description of the work completed in Year 3. Currently, the protocol has not been changed based on these analyses.

Sample Size Adjustment Consideration:

In order to help enrollment, we considered to reduce the sample size by dropping the CoQ10 arm. Revised sample size calculations were performed using the same study assumptions regarding the clinically meaningful detectable difference as well as the variability (Eidem, 2001). This analysis did not substantially reduce the total number of required participants due Because of the efficiency of the current factorial design for testing each of the two treatments' efficacy separately, these calculations showed only a modest decrease in required sample size that would result if the CoQ10 arm is dropped. A second calculation was performed using different underlying assumptions (Bahler, 2005). Resulting sample sizes for a two-group comparison of the efficacy of lisinopril were similar.

Exploration of Speckle Tracking Echocardiography:

We explored the potential of Speckle Tracking Echocardiography (STE), a novel echocardiography technique using natural acoustic reflections and interference patterns within an ultrasonic window, to evaluate whether it could quantify early changes in myocardial function in patients with muscular dystrophy. We evaluated its utility as an earlier marker of cardiac dysfunction with the goal of using it as inclusion criteria to identify patients whose cardiac function was considered normal by the present criteria but who showed evidence of cardiac dysfunction by STE.

We conducted a retrospective case control study of transthoracic echocardiograms of patients with DMD and an equal number of age and sex matched controls, using data from the echocardiography database at Children's National Medical Center (CNMC). A total of 33 boys with DMD (ages 7 -18 years old) with echocardiograms either performed at CNMC or available in the database from a larger CINRG multi-institutional study of cardiac outcome measures (see Section 2.3.4 above) during the period of January 2010 to June 2011 were included. Controls were identified as age-matched boys who had echocardiograms performed at CNMC but had no clinical cardiac findings and were assessed as normal echocardiograms. The study was approved by the institutional review board at CNMC.

In our preliminary analysis of these echocardiograms, STE demonstrated satisfactory left ventricular longitudinal tracking in 30 (91%) participants, strongly supporting the observation that STE technology can be applied to patients with muscular dystrophy. A comparison of STE data between DMD cases and controls showed that patients with DMD had significantly decreased global longitudinal and circumferential strain values ($p<0.001$). Of note, the control population cannot be considered entirely health controls as they were referred to the cardiology department at CNMC based on clinical complaints, and the findings on echo were negative; they were not necessarily healthy children from a general pediatric practice. We plotted longitudinal and circumferential peak systolic strain measures against ejection fraction (EF), the gold standard measurement of global systolic function, and while we found substantive variability, overall worse strain values (less negative) were evident with decreased EF. We compared the global longitudinal strain values in 30 patients with DMD and the corresponding myocardial performance index (MPI) to illustrate the effect of incorporating an STE-determined strain measure in the inclusion criteria for the study. Of these 30, if the study allowed inclusion of patients with a worse than -16 strain level, even if their MPI was <0.40, 13 additional patients would have met entry criteria and could have potentially been approached for study inclusion. However, STE is not a generally accepted criterion for cardiac function and some of the control subjects also had strain values worse than -16.

2.3.6 A longitudinal study of the relationship between impairment, activity limitation, participation and quality of life in persons with confirmed Duchenne muscular dystrophy protocol

A. Overview

There are two purposes to this study. The first purpose of this research study is to establish a large long-term assessment of people with DMD to better understand the current natural history of this disease, to be better able to design clinical trials based on ongoing natural history parameters. In this study, we are collecting data on participants' physical abilities across all ages, medical problems, and how they use healthcare services. We are also collecting data on how families of people with DMD interact with their communities and how they rate their quality of life. The second purpose of this study is to see how long-term steroid therapy affects these aspects of lives of participants with DMD.

This project is primarily funded by the Department of Education, which covers all patient related costs. In Year 3 we also received funding from the National Institutes of Health (NIH) for 2 ancillary studies discussed in the project update section below. For this project the activities covered on this grant relate to site monitoring, data management and statistical analysis activities.

B. Project Updates

Two ancillary studies were approved for funding by NIH early in Year 3. These studies were described in the previous annual report, prior to their funding. Briefly, one study adds novel clinical outcome measures and explores their feasibility, reliability, and validity in the DMD population, and their relationships to currently collected outcomes. This ancillary grant has also funded the addition of typically developing controls to generate a set of normal predicted values for strength and function assessments. The second ancillary study focused on biomarkers. It is also anticipated that funding will be received for enrolling a second cohort of younger DMD patients to enrich the data set and also to explore biomarker changes in the presence of initiating glucocorticoid treatment. The study team dedicated much of their efforts to the finalization of this complex protocol amendment based on all 3 of these additional studies and removing some outcomes on which sufficient information was already collected. This amendment includes revised and updated consents and assents. This protocol amendment approved by the institutional review board (IRB) overseeing the CC as well as the IRB overseeing the study chair at University of California, Davis.

C. CINRG Site Updates and Site Monitoring

In Year 3, the project management team performed 10 site monitoring visits. The following tasks are performed during each monitoring visit:

- Review of protocol conduct and adherence to regulatory guidelines
- Source document verification, including the review of informed consent documents and adverse event/serious adverse events
- Review of outstanding queries
- Review of strength and functional testing equipment and space
- Protocol training for any new staff
- Re-training of any identified areas of inconsistency or concern

Below is a summary of the findings from each completed on-site monitoring visit conducted since in Year 3:

- University of Tennessee in Memphis, TN: From January 22nd – 25th, 2012, the operations manager and a newer project manager from the University of Pittsburgh site completed a combined visit to initiate the site with the PITT0908 Clinical Trial (see Section **2.3.5**) and monitor their activities in this study. Although the site is well functioning, there were several issues relating to data completion. The CC developed a tight follow-up plan to resolve all pending items from the monitoring visit with the site staff. The site staff was also re-educated on good clinical practice and research guidelines.

- The Royal Children's Hospital in Melbourne, Australia: From February 10th – 15th, 2012, the operations manager completed a site monitoring visit. The site was found to be functioning very well. The site was found to have very few outstanding data queries and their overall study compliance and documentation was in good order.
- The Children's Hospital of Westmead in Sydney, Australia: From February 16th – 17th, 2012, the operations manager completed a site monitoring visit. The study records and data quality was in very good order, but there were some lapses in regulatory processes and documentation. The CC developed a plan with the site to close all gaps.
- Alberta Children's Hospital in Calgary, Canada: From June 18th – 22nd, 2012, the operations manager completed a combined visit to monitor the PITT0908 Clinical Trial (see Section **2.3.5**) and monitor their activities in this study. This site was found to be functioning well. The site was found to have few outstanding data queries and their overall study compliance and documentation was in good order.
- University of Puerto Rico in San Juan, PR: From June 28th – 29th, 2012, the operations manager completed a site monitoring visit. The site staff has experienced some difficulties with keeping their participants engaged in the study, and long term follow-up visits are missing. The CC developed a plan with the site to contact their participants.
- Children's National Medical Center in Washington, DC: From June 12th – 21st, the new project manager from the CINRG CC completed a site monitoring visit. This site is currently undergoing some reorganization of staff. Some discrepancies were noted surrounding the consent process as the previous site staff re-consented their participants at annual visits, which was not needed. The study records and data quality was in good order.
- Fundacion Favaloro in Buenos, Argentina: From July 2nd – 6th, 2012, the operations manager completed a site monitoring visit. This site was found to be functioning very well. There were a few outstanding data queries, all of which were resolved during the visit.
- University of Pittsburgh in Pittsburgh, PA: From July 11th – 12th, 2012, the operations manager and the new project manager from the CINRG CC completed a combined visit to monitor the PITT0908 Clinical Trial (see Section **2.3.5**) and monitor their activities in this study. The site was found to be functioning very well. The site was found to have very few outstanding data queries and their overall study compliance and documentation was in very good order.
- Holland Bloorview Kids Rehab in Toronto, Canada: From July 16th – 19th, 2012, the operations completed a site monitoring visit. The site was found to be functioning very well. The site was found to have very few outstanding data queries and their overall study compliance and documentation was in good order.
- University of California, Davis in Sacramento, CA: From August 13th – 17th, 2012, the operations and the new project manager form the CINRG CC completed a site monitoring visit and training for the new protocol amendment. The main findings were that some case report forms had not been sent to be scanned into the dataset, creating

a delay in the data completion. The site staff resolved the submission of all pending case report forms during the site monitoring visit. The new protocol amendment training session was very well received by the entire site staff. This site, which is the lead study site, is now the first site set-up to complete all updated assessments per the new protocol amendment.

D. Data Management

The data management team has continued to issue data checks to each site to ensure collected data are accurate and reliable.

In preparation for each monitoring visit described above the data management team prepared a comprehensive report of data summaries and a detailed list of any outstanding data queries to aid the project management team in monitoring.

With the new protocol amendment the data management team has been preparing the transition of the case report forms from paper scanable Teleforms to electronic data capture with OpenClinica. This was an opportunity to both update forms to capture all required data in the amendment and make some minor improvements based on insight from managing data from this study for the past few years. This effort included a collaboration of the entire CC team as well as the study leadership in UC Davis.

In the transition of the database the patient surveys were also converted to allow online completion by the participants and/or caregiver to keep with current technology and its use by the general population. The online surveys are in REDCap (http://www.project-redcap.org). The data from OpenClinica and REDCap will be linked by study participant ID.

E. Statistical Analysis

In Year 3 analyses were performed for 5 draft manuscripts.

- Updated analyses to complete the submission of two manuscripts, one describing the study methods and the second describing baseline and some first study year results manuscripts (see **Key Accomplishments**) were performed.
- Analyses were performed for a platform presentation and subsequent preparation of a draft manuscript on height findings in DMD. Using growth charts from Center for Disease Control (CDC), we categorized whether the participants met the criteria for short stature. We explored the relationship between height, ambulatory status, steroid status, and years on steroid. Both observed and calculated (based on ulnar bone length) height were used in the analyses. Statistical techniques used included Fisher’s Exact test, and multiple logistic and linear regression modeling.
- To assist the strength and function manuscript working group in their development of several planned manuscripts analyses were performed using both baseline data and longitudinal data. Scatter plot was used to explore the correlation between various components of muscle strength. Each muscle strength component was summarized by age group and steroid status as well as their changes from baseline. Survival analyses were performed to predict the probability of ambulation after study entry within groupings based on timed function tests at study entry.
- Analyses were performed for a secondary publication on genetic modifiers of DMD. The DMD Natural History Study dataset was utilized to evaluate the effect of a single nucleotide polymorphism in the osteopontin (SPP1) gene (-66 T>G -rs28357094) on longitudinal functional measures. Saliva samples for DNA genotyping were available for 280 DMD participants. The outcomes of interest in the longitudinal follow-up were

age at loss of ambulation and the time to run or walk 10 meters. Participants were stratified into 2 groups according to a dominant genetic model (TT homozygotes versus GT heterozygotes and GG homozygotes). Comparisons between genotype groups were performed using both standard methods, and averages within age groups with moving overlapping age groups as well as mixed effects linear models.

F. Manuscript Preparation

The main methodology/baseline results manuscript was submitted in the early part of Year 3 and was reviewed with several comments. In order to address these comments the study team separated the manuscript into two manuscripts. Those are “The CINRG Duchenne Natural History Study – A longitudinal natural history study in the era of glucocorticoid therapy: Design of the protocol and methods” and “The CINRG Duchenne Natural History Study: Glucocorticoid treatment preserves clinically-meaningful functional milestones and reduces rate of disease progression as measured by manual muscle testing and other commonly used clinical trial outcome measures”. These two manuscripts were resubmitted and are currently in review.

Draft manuscripts are currently circulating among co-authors for the genetic modifier using data, the cardiac data, and the height data, all described above.

2.3.7 Becker Muscular Dystrophy – A Natural History Study to Predict Efficacy of Exon Skipping

A. Overview

This new project’s objective is to phenotype participants with in-frame mutations of the dystrophin gene, corresponding to target deletions generated by skipping exons 45, 51 and 53. This information will then be used to integrate phenotype information by severity associated with each deletion. This study will also evaluate potential clinical trial outcome measures for participants with Becker muscular dystrophy. The study period is 36 months per patient. This project is primarily funded by the National Institutes of Health (NIH).

B. Project Updates

In the first year of NIH funding the clinical protocol was developed and approved by the CINRG Executive Committee (EC) and CINRG DSMB. The study was also submitted and approved at the IRBs of the CINRG CC and the CINRG central site at the University of Pittsburgh. The study team also developed the accompanying MOOP and case report forms. The data management team developed the case report forms in OpenClinica. The database was tested by all lead members of the CINRG CC in order to identify any errors prior to finalizing the study database.

2.3.8 Duchenne Muscular Dystrophy Tissue Bank for Exon Skipping

A. Overview

This new project will create the first DMD Tissue Bank that will collect tissue and blood from DMD participants with specific genetic mutations within the dystrophin gene that could be treated by anti-oligonucleotide drugs. The DMD Tissue Bank will validate dystrophin mutations and provide a single, comprehensive, organized collection of properly prepared and retrievable de-identified fibroblast cell cultures and blood samples to be used for current and future research studies in muscular dystrophy for exon skipping research strategies. This project is primarily funded by the National Institutes of Health (NIH).

B. Project Updates

In the first year of NIH funding the clinical protocol was developed. The laboratory technician developed the laboratory MOOP and validated all procedures and storage with three participants' tissue and blood samples.

2.4 CINRG Administrative Efforts

2.4.1 CINRG CC Team Meeting

The CINRG CC continued to hold weekly team meetings. The CINRG CC team discusses protocol progresses and infrastructure related updates on alternating weeks. Protocol related meetings are attended by all members of the CINRG CC along with the CINRG study chairs.

2.4.2 CINRG 2012 Membership and Scientific Meeting

The CINRG CC has been planning the November 2012 Membership and Scientific Meeting. A contract was established with the venue and subsequent planning meeting have occurred to organize the logistics of the meeting. The program committee has also met and generated the preliminary programs for the CINRG Membership meeting as well as the Scientific meeting.

2.4.3 CINRG Executive Committee Meetings

The CINRG EC is responsible for, among other things, the review/approval of all protocols to be conducted by the network/utilize CINRG equipment; oversee programmatic activities of CINRG, and assess or implement recommendations from CINRG's Scientific Advisory Committee (SAC).

The CINRG Operation Manager coordinates the dissemination of necessary documentation and review/voting conduct of this committee. The committee has conducted 3 meetings, including the review of 2 new CINRG network sites, review/approval of 2 new protocols, and continued oversight of CINRG activities and conduct.

2.4.4 CINRG Network Communication

The CINRG CC maintains formal communication and provides network updates to participating sites through periodic teleconferences. These teleconferences are sub-divided into two formats; one to accommodate site principal investigators and another to accommodate clinical coordinators and evaluators. In Year 3 a total of 3 meetings were held.

The CINRG website also provided CINRG members with a means of communication. The CC continues to upload necessary documents on the private section to communicate with the CINRG sites. The CC also continues to work with the vendor to improve the look and features of the private site.

2.4.5 Collaborating with Other DMD Research Entities

In Year 3 several members of the CINRG CC have attended meetings with other DMD research entities and the CC continues their active collaborations with the DMD community.

- The operations manager participated in the TREAT-NMD 2011 Conference from November 8th – 11th, 2012 in Geneva, Switzerland. This meeting brought together over 250 delegates with an interest in neuromuscular disorders. The operations manager presented two posters on behalf of CINRG and the DMD Natural History Study (see **Key Accomplishments**). This meeting also preceded the TREAT-NMD

Global Database Oversight Committee meeting for which the operations manager is a committee member.

- The CEM continued to work with TREAT-NMD to harmonize clinical outcome measures and standardize manuals of operations for current and future test measures. To date, the updated manual for several of our CINRG studies have standardized operational definitions and instructions that are consistent with protocols reviewed and implemented by the TREAT-NMD network. From September 14^{th} – 16^{th}, 2011, the CEM was certified at a meeting in Boston to train CEs on one of the new functional assessments North Star Ambulatory Assessment (NSAA). The NSAA is a functional assessment that is validated in boys with DMD and used in large multi-center studies around the world (Mazonne et al, 2009). This NSAA certification has allowed the CEM to train other CINRG CEs who have not previously received NSAA training.

  The CEM has also been involved with an international working group of physiotherapists as part of the DMD Outcomes Working Group to analyze and develop an upper limb functional strength measure called Performance of Upper Limb (PUL) Scale for individuals with DMD. This scale was developed based on Rasch analysis of items currently used in DMD outcomes such as the Brooke Upper limb scale, NSAA, Hammersmith (Krosschell et al, 2006), and the motor function measure (MFM, Diniz, 2012). The scale combines functional movements with a graded strength component that takes into account activities of daily living. All items on this scale relate to an activity typically performed by boys with DMD.

  Two meetings of expert physiotherapists, which included 2 experts from the United States, one of whom is the CINRG CEM, were held in Newcastle from January 10^{th} – 12^{th}, 2012 and March 26^{th} - 27^{th}, 2012 to develop and test the PUL. In parallel to this group, a separate focus group developed a Patient Reported Outcome Measure to assess quality of life with input from patients and families affected by DMD.

  Two multidisciplinary meetings that included twenty members from parent organizations, neuromuscular physicians, and industry, and included the CINRG CEM, took place in Rome on February 15^{th} – 16^{th}, 2012 and July 20^{th} – 22^{nd}, 2012. Accomplishments from the meetings include finalizing the 2 newly developed outcome measures to ensure agreement between items and clinical meaningfulness. A workshop report was submitted for publication following the February meeting. A poster presentation of the preliminary results of PUL was accepted and will be presented at the World Muscle Society in October 2012.

- Collaboration and participation in TACT review. CINRG CC’s director collaborates with TREAT-NMD by serving as the statistician reviewer for the TREAT-NMD Advisory Committee for Therapeutics (TACT, http://www.treat-nmd.eu/resources/tact/reviews/past). TACT is an expert multidisciplinary body that provides the neuromuscular community (clinicians, researchers, patient advocacy groups and industry) with independent and objective guidance on advancing new therapies for neuromuscular diseases. TACT provides an in depth review which helps therapeutic developers in various aspects of the drug development process. The CINRG CC director provided input in four reviews on issues of clinical study design, feasibility and other clinical research considerations, statistical interpretation of previous study results (preclinical or clinical) leading to the current interpretation, and statistical analyses plans reviews for proposed studies.

- In June 2012 the CINRG Medical and Scientific Directors were invited by the National Institute for Neurological Diseases and Stroke to provide feedback on their ongoing efforts to develop Common Data Elements (CDE) for neuromuscular disease research. The CINRG CC team divided up the 7 review domains amongst the team based on expertise. The CINRG CC submitted their feedback as a unified group on July 31st, 2012.
- The CINRG Medical Director, Dr. Clemens, was a participant in the PPMD-sponsored conference ‘Transforming Duchenne Care’ from June 27-28, 2012, held in Ft. Lauderdale, FL in conjunction with the PPMD CONNECT 2012 Annual Conference. The participants in the meeting included parent, physician, medical center administrator, and foundation member stake holders. The focus of the meeting was to understand currently available models of care for DMD patients at all stages of disease and ages, and to use this as a base to brainstorm about the transformation of the model of care to better serve patients and their families. The meeting included large group discussions, presentations and small focus group discussions.

2.4.6 Infrastructure Subcontracts

The CINRG CC has maintained their subcontracts with seven sites, and one consultant agreement, to provide support for site research related activities, including regulatory duties, attendance at CINRG meetings, conferences and training, including training of CE staff on the CQMS.

The subcontract with OpenClinica was also continued and a new contract was established for the clinical trial management system, Evolve (see section **2.4.7**).

2.4.7 CINRG Regulatory Compliance Assurance

The project management team continues to work with each CINRG site to assure ethical and regulatory compliance for each related protocols.

A. Ethics Submission Assistance

The CC continues to provide assistance with ethics application packets to all participating sites for each related protocols. The project management team has maintained regular contact with the sites to assist with the preparation of the submission documents. All informed consents and assents were reviewed by the study project manager before they were submitted to their respective ethics committees. All sites received assistance until protocol and consent/assent documents received local approval.

B. Assurance of Regulatory Compliance

The project management team continues to be responsible for ensuring that every site has their regulatory documents up to date. In Year 3, a new Clinical Trial Management System called Evolve (https://se44sl2.studymanager.com) has been purchased with funds outside this award to facilitate the collection and tracking of all site regulatory and personnel documents. With a proven workflow and intuitive user experience, Evolve simplifies the day-to-day management of clinical trials. Evolve is maintained externally by a vendor and is a secure and very well backed up system. Evolve is structured to allow for several hierarchies to track studies, sites, personnel, equipment, and monitoring visits:

- Study Level: This level allows the CC to track essential central study documents such as the multicenter protocol, template consents, case reports forms.

- Site Level: This level allows the CC to track essential site specific documents such as the site specific ethic approvals, site approved consents, site approved continuing reviews.
- Personnel Level: This level allows the CC to track essential personnel documents such as curriculum vitae, medical licenses, human subject training certificates
- Equipment Level: We have designed a study specific project that will allow the CC and CEM to track supplies across all of our studies and sites.
- Monitoring Visit Level: This level allows the CC to complete monitoring reports electronically and track items across visits and studies

In Year 3, we worked with the Evolve team to set up training modules for all the levels described above as well as creating a custom report that will allow the CC to run reports to monitor any upcoming deadlines for ethics renewals, human subject training certificates, medical licenses, and more. The training sessions were conducted from May 11th through June 20th, 2012.

To date, the CINRG CC has entered all personnel documentation for the CINRG sites and has assigned project specific entry amongst the project management team and CEMs. Many of the documents were uploaded to the system.

2.4.8 CQMS Equipment and Supplies Summary

CINRG has utilized the previously developed supplies pamphlet to perform annual supplies and instrument checks to ensure quality of testing instruments. The CEM has also established contracts with individual vendors to obtain necessary supplies for maintenance of items required for implementation of CE assessments. In this past year, CINRG implemented the new mechanism for obtaining supplies for new CINRG sites. Since the instrument and supplies pamphlet has detailed information of the manufacturer and serial number of the standardized supplies used by CINRG, new sites are able to use their institution's buyers to order some of the items required for standardized CQMS testing.

The site in Tokyo has obtained all supplies through this new process and two sites (in Kobe, Japan and at Duke University) have identified supplies needs in which the CEM will assist in ordering the appropriate items for site set up.

We are working on a new standard operating procedure for site initiation and will utilize the supplies and instrument pamphlet and a newly developed supplies screening process that will best utilize resources from both CINRG and the institution to identifying possible shared costs for better utilization of funds.

The CEM has started to utilize the clinical trials management system, Evolve, to effectively track supplies for the CINRG network. This system improves oversight of instruments and supplies for current and upcoming requirements in CINRG studies.

2.4.9 CINRG Subcommittee Updates

<u>CINRG Scientific Advisory Committee (SAC)</u>: The SAC is a committee whose aim is to set research priorities and offer operational recommendations to the CINRG CC and routinely convenes during the CINRG Investigator meeting. The SAC is scheduled to meet during the upcoming November 2012 Meeting.

<u>CINRG Publication Subcommittee (CPS)</u>: In Year 3, the CPS has received 14 review requests:

- Six abstract review requests

- Five academic meeting presentations requests broken down as two posters and three platform presentations
- Two data summary requests
- Three manuscript review requests

CINRG Therapeutic Subcommittee (CTS): The broad role of the CTS is to undertake an active role of bringing potential agents for evaluation towards clinical trials utilizing the CINRG network. In Year 3, the CTS did not hold meetings.

CINRG Outcomes Subcommittee (COS): The broad role of the COS is to undertake an active role of review outcomes for studies in the CINRG network. In Year 3, the COS has met 5 times. They provided recommendations for the outcomes in the latest protocol amendment of the DMD Natural History Study and provided recommendations for the new BMD Natural History Study.

CINRG Data Safety Monitoring Board (DSMB): The DSMB is responsible for safety monitoring and monitoring of data integrity for all CINRG studies. The DSMB includes neurologists, patient advocates, and a statistician. The DSMB held an annual meeting on September 7, 2011 and the following studies were discussed:

- PITT0908 – Clinical Trial of Coenzyme Q10 and Lisinopril in Muscular Dystrophies
- A Longitudinal Study of the Relationship between Impairment, Activity Limitation, Participation and Quality of Life in Persons with Confirmed Duchenne Muscular Dystrophy (DMD)
- Evaluation of Limb-Girdle Muscular Dystrophy
- Cardiac Outcome Measures in Children with Muscular Dystrophy
    - ECHO Protocol: PITT1109 – Cardiac Outcome Measures in Children with Muscular Dystrophy
    - Cardiac MRI Protocol: PITT0110 - Cardiac Magnetic Resonance: A Parallel Protocol to Cardiac Outcome Measures in Children with Muscular Dystrophy

The DSMB noted concerns about reaching enrollment goals for the CoQ10 Lisinopril Trial but all studies were approved to move forward according to protocol.

Because the CoQ10 Lisinopril Trial involves a study drug the DSMB also held a second meeting on July 25, 2012. The CINRG study team presented multiple areas they have researched to either lower the sample size or improve enrollment as discussed in section **2.3.5** above. The DSMB approved for the protocol to continue with the consideration of removing Month 3 and 9 to reduce patient burden.

3. Key Accomplishments

The following are a summary of the key accomplishments for the Year 3 funding period:

- One manuscript was published (see attached **Appendix**)
- Sixteen CINRG site visits were completed: five for CEM training only, eight for project management monitoring only, and three as combination CEM and project management monitoring
- Two new project managers were trained to complete monitoring visits according to CINRG standards
- One new CEM was trained to complete CE certifications and re-certifications
- Eleven new clinical evaluators were trained
- Ten existing clinical evaluators were re-certified
- Two new protocols were developed

4. Reportable Outcomes

The following are a summary of the reportable outcomes for the Year 3 funding period:

- Manuscript citation:
  - Escolar DM, Zimmerman A, Bertorini T, Clemens PR, Connolly AM, Mesa L, Gorni K, Kornberg A, Kolski H, Kuntz N, Nevo Y, Tesi-Rocha C, Nagaraju K, Rayavarapu S, Hache LP, Mayhew JE, Florence J, Hu F, Arrieta A, Henricson E, Leshner RT, Mah JK. Pentoxifylline as a rescue treatment for DMD: a randomized double-blind clinical trial. *Neurology* 2012 Mar 20;78(12):904-13.

- Informatics:
  - Implementation of the clinical trial management system: Evolve
  - NIC Software CQMS3 alpha testing

- Funding applied for based on work supported by this award:
  - Submission of a PPMD grant entitled “Clinically Meaningful Outcomes for Duchenne Muscular Dystrophy Therapeutic Trials in 100 additional young DMD boys” in November 2011
  - Submission of a DOD grant entitled “Establishing minimal clinically important differences for current clinical trial endpoints and composite outcome measures in Duchenne muscular dystrophy via extension of a multicenter natural history” in January 2012
  - Submission of an NIH Center Grant (P60) entitled “Muscular Dystrophy Multidisciplinary Clinical Research Center” in May 2012
  - Submission of an American Heart Association (AHA) Grant entitled “Pilot trial of losartan in young patients with Duchenne muscular dystrophy” in July 2012

5. Conclusion

The infrastructure support for CINRG’s CC has continued in Year 3 to be an invaluable resource to the network and to the neuromuscular community. Four strong new studies were proposed, at least two of which are in process of being implemented, and two are still under review. A complex protocol amendment to the ongoing DMD Natural History Study was approved and is being disseminated through the network. This amendment adds important information to an already rich and pivotal study by implementing novel clinically meaningful outcome measures, adding control participants, and collecting samples for biomarker data. Two new protocols that will provide critical information and resources for exon skipping treatment in muscular dystrophies were also developed during this past year. The CINRG CC continued to support all participating clinical sites by completing sixteen on-site visits. These visits provided sites with hands-on training of new site personnel as well as on-going monitoring of studies. The CINRG network sustained their leadership role within the neuromuscular community by actively participating and contributing to diverse projects such as TREAT-NMD, parent foundations, and NIH common data elements. Finally, CINRG published results from a previous study and is positioned to submit additional manuscripts on several recently completed projects.

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Appendix

- Published manuscript

Neurology®

Pentoxifylline as a rescue treatment for DMD : A randomized double-blind clinical trial

D.M. Escolar, A. Zimmerman, T. Bertorini, et al.

Neurology 2012;78;904; Published online before print March 7, 2012;
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This information is current as of September 13, 2012

Pentoxifylline as a rescue treatment for DMD

A randomized double-blind clinical trial

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ABSTRACT

Objective: To determine whether pentoxifylline (PTX) slows the decline of muscle strength and function in ambulatory boys with Duchenne muscular dystrophy (DMD).

Methods: This was a multicenter, randomized, double-blinded, controlled trial comparing 12 months of daily treatment with PTX or placebo in corticosteroid-treated boys with DMD using a slow-release PTX formulation (~20 mg/kg/day). The primary outcome was the change in mean total quantitative muscle testing (QMT) score. Secondary outcomes included changes in QMT subscales, manual muscle strength, pulmonary function, and timed function tests. Outcomes were compared using Student *t* tests and a linear mixed-effects model. Adverse events (AEs) were compared using the Fisher exact test.

Results: A total of 64 boys with DMD with a mean age of 9.9 ± 2.9 years were randomly assigned to PTX or placebo in 11 participating Cooperative International Neuromuscular Research Group centers. There was no significant difference between PTX and the placebo group in total QMT scores ($p = 0.14$) or in most of the secondary outcomes after a 12-month treatment. The use of PTX was associated with mild to moderate gastrointestinal or hematologic AEs.

Conclusion: The addition of PTX to corticosteroid-treated boys with DMD at a moderate to late ambulatory stage of disease did not improve or halt the deterioration of muscle strength and function over a 12-month study period.

Classification of evidence: This study provides Class I evidence that treatment with PTX does not prevent deterioration in muscle function or strength in corticosteroid-treated boys with DMD.
Neurology® 2012;78:904–913

GLOSSARY

AE = adverse event; **CINRG** = Cooperative International Neuromuscular Research Group; **CTCAE** = Common Terminology Criteria for Adverse Events; **DMD** = Duchenne muscular dystrophy; **MMT** = manual muscle testing; **NCI** = National Cancer Institute; **PFA** = platelet function assay; **PFT** = pulmonary function test; **PT** = prothrombin time; **PTT** = partial thromboplastin time; **PTX** = pentoxifylline; **QMD** = quantitative muscle testing; **TGF-β** = transforming growth factor-β; **TNF-α** = tumor necrosis factor-α.

Duchenne muscular dystrophy (DMD) is the most common type of muscular dystrophy of childhood caused by mutations involving the dystrophin gene. The loss of dystrophin leads to muscle membrane fragility, altered calcium homeostasis, and increased oxidative stress, which in turn triggers a cascade of pathologic events that ultimately results in muscle necrosis, fibrosis, and impaired muscle regeneration.[1,2] Corticosteroids are currently the only available disease-modifying therapies for DMD, by prolonging independent ambulation and delaying the onset of secondary complications.[3–5] However, the use of chronic high-dose corticosteroids for DMD is frequently associated with significant side effects and does not halt disease progression. An

Supplemental data at www.neurology.org

Supplemental Data

From the Children's National Medical Center (D.M.E., A.Z., C.T.-R., K.N., S.R., J.E.M., F.H., A.A., E.H., R.T.L.), Washington, DC; Departments of Neurology and Pathology (T.B.), University of Tennessee Health Sciences Center, Memphis; University of Pittsburgh and Department of Veterans Affairs Medical Center (P.R.C.), Pittsburgh, PA; Washington University in St. Louis (A.M.C.), St. Louis, MO; Instituto de Neurociencias (L.M.), Fundación Favaloro, Buenos Aires, Argentina; Child Neurology and Psychiatry Department (K.G.), IRCCS C Mondino Foundation, Pavia, Italy; Royal Children's Hospital (A.K.), Melbourne, Australia; University of Alberta (H.K.), Edmonton, Canada; The Mayo Clinic (N.K.), Rochester, MN; Hadassah Hebrew University Hospital (Y.N.), Jerusalem, Israel; and University of Calgary (J.K.M.), Calgary, Canada.

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Study funding: Funding information is provided at the end of the article.

Disclosure: Author disclosures are provided at the end of the article.

effective treatment for DMD may require a combination of therapies, including pharmacologic agents and gene or cell-based approaches targeting different pathways involved in muscle necrosis and degeneration.[6,7]

Pentoxifylline (PTX) is a phosphodiesterase inhibitor with potential ability to counteract the complex pathology in DMD; it improves calcium homeostasis and diminishes inflammation, fibrosis, and oxidative stress.[8,9] Preclinical studies showed that PTX reduced muscle strength deterioration by 51% in the exercised *mdx* mouse.[10] The anti-inflammatory effect of PTX is mediated primarily through the inhibition of tumor necrosis factor-α (TNF-α) production by adenylyl cyclase activation and increased cellular cyclic AMP.[11–13] In addition, PTX decreases fibrosis by altering the metabolism of metalloproteinases and collagen through the transforming growth factor-β (TGF-β) pathways that are upregulated in DMD.[14–16] In addition, PTX has antioxidant effects by inhibiting polymorphonucleated cell degranulation and improves peripheral circulation by increasing red blood cell deformability, reducing blood viscosity, decreasing platelet aggregation, and increasing blood glucose supply.[17] The combination of PTX plus corticosteroids had a synergistic and profound immunomodulatory effect on stimulated human peripheral blood mononuclear cells.[18] However, the clinical benefits of PTX in children with DMD remained unknown.

As part of an initial exploratory study, our group conducted an open-label pilot study of an immediate-release oral formulation of PTX at 20 mg/kg/day in 17 corticosteroid-naive boys between 4 and 8 years of age with DMD.[19] Although there was no observable deterioration in strength or motor function over a 12-month treatment period, the immediate-release formulation of PTX was poorly tolerated because of significant gastrointestinal side effects and/or neutropenia, thus precluding adequate assessment of efficacy. The lack of deterioration in that small group of patients with DMD, however, was encouraging and prompted the current study. To circumvent the gastrointestinal side effects, we used a Food and Drug Administration–approved slow-release formulation of PTX in this study as an add-on treatment to corticosteroids to determine the benefits of combination therapies for boys with DMD over those of corticosteroids alone. We also included additional laboratory monitoring as safety measures to watch for early signs of neutropenia or other adverse events (AEs) related to PTX. We hypothesized that a 12-month treatment of daily oral PTX in corticosteroid-treated boys with DMD would result in significantly increased muscle strength as measured by their total quantitative muscle testing (QMT) scores compared with scores of those treated with corticosteroids alone.

METHODS

Trial design. This was a randomized, multicenter, double-blinded, placebo-controlled trial conducted in 11 academic institutions that are members of the Cooperative International Neuromuscular Research Group (CINRG) from September 2005 to January 2008. Ambulant boys with DMD were randomized in a parallel equal (1:1 ratio) allocation to either oral PTX or placebo.

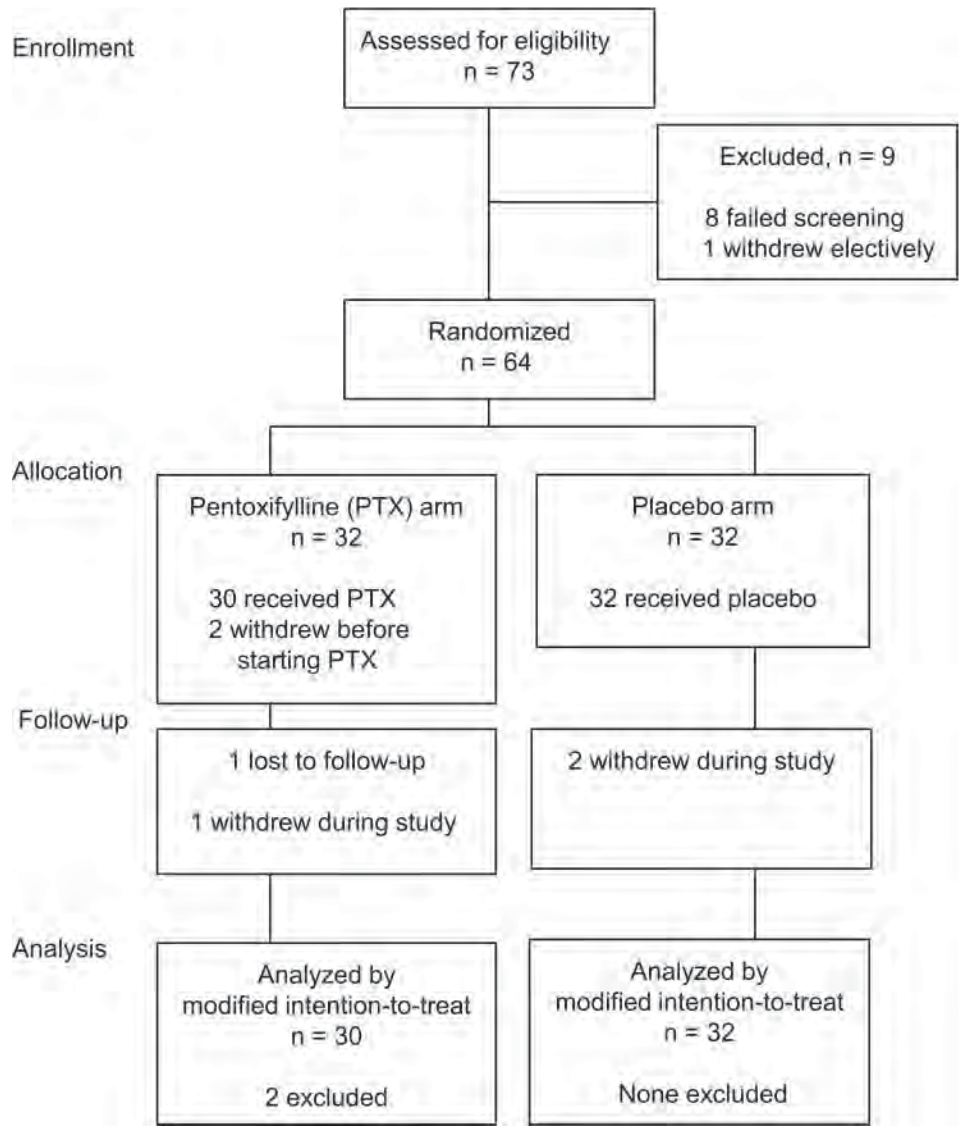
Study participants. Ambulant boys ages 7 or older with a confirmed diagnosis of DMD were recruited. Participants were required to be taking a stable dose of corticosteroids (either prednisone or deflazacort) for at least 12 months before screening, with normal blood clotting ability based on platelet function assays (PFAs). In addition, participants had to demonstrate consistent muscle testing efforts between screening visits (maximum 7 days apart) with no more than 15% variation in a unilateral biceps QMT score before enrollment. Participants were excluded from participation if they were currently participating in another clinical trial, had a recent cerebral or retinal hemorrhage, or had a history of significant concomitant illnesses including renal or hepatic impairment, bleeding diathesis, or gastric ulcer.

Standard protocol approvals, registrations, and patient consents. All participating clinical research centers obtained approval from their local institutional research board or ethics review board. This study was registered at the NIH Web site (accessed via www.clinicaltrials.gov, registration number NCT00243789). Each participant gave assent, and the parents provided written informed consent as consistent with local institutional policy before the conduct of any study-related assessments.

Interventions. ***Study arms.*** Participants were randomly assigned to receive either daily oral PTX or placebo while continuing preexisting corticosteroid therapy at a stable dose. With the exception of multivitamins, vitamin D, and calcium, use of nutritional supplements was not permitted during the study.

Study drug. The study drug was PTX (Trental; Sanofi-Aventis U.S. LLC, Bridgewater, NJ) tablets, an FDA-approved pharmaceutical that is available for oral administration as 400-mg oblong tablets. Both the study drug PTX and placebo were overencapsulated by Capsugel (Pfizer Inc.), a clinical trial

Figure 1 Participant flow through the trial



grade opaque gelcap that is supplied by Fischer Pharmaceuticals. Each PTX capsule contained one 400-mg time-release PTX tablet and inert filler. The placebo capsules contained only inert filler.

Dosing. Participants received 1 of 3 dosing regimens based on their weight at the screening visit: those weighing less than 30 kg received 400 mg once/day; those weighing between 30 and 50 kg received 400 mg twice/day; and those weighing greater than 50 kg received 400 mg 3 times per day, not to exceed 20 mg/kg/day or total dose of 1,200 mg/day, according to available safety information in the pediatric population.[20–23]

Criteria for dose reduction. During the study, a dose reduction by 400 mg/day was performed if participants experienced AEs; doses were not re-escalated. Criteria for dose reductions included an increase in prothrombin time (PT), partial thromboplastin time (PTT), or PFA over the upper limit of normal, or any grade 4 severe AE as defined by National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) grading criteria (version 3.0, available via http://ctep.cancer.gov). Safety data and all AEs were reviewed by the CINRG Data and Safety Monitoring Board on a regular basis.

Study procedures and evaluation. Participants had a total of 9 study visits. At screening, 2 QMT assessments were performed (up to 7 days apart for reliability inclusion), then a safety blood draw at day 15, and further evaluations at study treatment months 1, 3, 6, 9, and 12. Participants had a final QMT assessment 1 week after the end of treatment (figure 1). At each study treatment visit, clinical and safety evaluations included review of medical history, medication administration records, pill counts, AE collections, physical examination, and strength and function assessments as measured by the CINRG quantitative measurement system, platelet function assessment (PFA-epinephrine and PFA-adenosine diphosphate), and standard laboratory panels including complete blood count, PT, PTT, serum chemistry, creatine kinase, electrolytes, glucose, total cholesterol, renal, and liver function tests.

Study outcomes. The primary endpoint was the change in mean QMT total scores from baseline to the end of treatment. The total QMT score (measured in pounds) is the average force generated from 10 muscle groups (including bilateral elbow flexors, elbow extensors, knee flexors, knee extensors, and hand grip), as measured by the CINRG quantitative measurement system.[24,25]

The secondary endpoints included change in mean QMT scores for arm, leg, grip,[24,25] elbow extensors, elbow flexors, knee extensors, and knee flexors. Other secondary endpoints included change in velocity of timed function tests to walk 10 m, climb 4 stairs, and stand from supine, manual muscle testing (MMT) scores,[26] Brooke upper extremity[26] and Vignos lower extremity[27] functional rating scales, goniometry measurements,[26] and pulmonary function tests (PFTs).[28] Pediatric health-related quality of life was assessed using the PedsQL version 4.0 parent-report form.[29,30] Surrogate outcome measurements of TGF-β and TNF-α as markers of fibrosis and inflammation were also included as exploratory endpoints. TGF-β1 and TNF-α cytokines were measured using ELISA kits (88-7344 and 88-7346; eBioscience, San Diego, CA).

Statistical methods. ***Sample size estimation.*** The sample size was calculated based on the mean total QMT scores obtained from 50 boys aged 4–10 years with DMD who were enrolled in a previous CINRG trial.[19] Twenty-eight participants in each arm allowed us to detect a difference in total QMT score between groups of 1.0 (SD 1.3) pounds, which was considered clinically significant, with 80% power and a 2-sided α of 0.05. The sample size was increased to 32 participants in each of the 2 arms to allow for up to 20% participant withdrawals or incomplete follow-up and for one interim analysis at 6 months.

Randomization and blinding. Patients were randomly assigned using a randomly permuted balanced blocks of variable size (2 and 4) approach. The CINRG coordinating center generated the randomization schedule and provided it to the central pharmacist. The central pharmacist was responsible for dispensing and shipping the supply of investigational drug to the study sites for the duration of treatment. Each site dispensed the study medications to the participants and kept an accurate medication log. All participants, site principal investigators, clinical evaluators, and study coordinators were blinded to the group assignments.

Statistical analysis. Baseline participants' characteristics were summarized using means and SDs. The two-sided Student t test was used to compare the changes in the total QMT scores at 12 months between PTX and placebo groups. All randomly assigned participants who received one or more doses of study medication were included in accordance with the modified intention-to-treat analysis; only participants who withdrew before receiving any treatment were not included. To carry out the intention-to-treat approach, a single imputation was used to impute the 12-month values if the assessments were not done at that time because of dropout or any other reasons except for disease progression. Participants who were unable to perform selective components of the QMT because of disease progression were assigned a total QMT score of 0. To investigate the change in QMT scores and timed function tests over time, a linear mixed-effects model was constructed to account for the correlation between the observations due to the clustered structure of the data.[31] Comparisons between the components of the linear evolutions (intercepts and slopes) were performed using the F-test.

The secondary outcomes between the 2 study arms including changes in arm, leg, grip, elbow extensors, elbow flexors, knee extensors, and knee flexors QMT as well as the total MMT, velocity of timed tests, Brooke and Vignos functional rating scales, degree of joint contractures, PedsQL scores, and PFTs were compared using 2-sample t tests.

The frequency, body system, severity, and relationship to drug of AEs were tabulated for each group according to CTCAE categories and compared using the Fisher exact test. Analyses were performed using SAS/STAT software 9.1; $p < 0.05$ was considered to be statistically significant.

RESULTS **Recruitment and baseline data.** Eleven participating CINRG institutions screened a total of 73 boys, of whom 65 were eligible and 64 were enrolled and randomly assigned in equal numbers to the 2 study arms. Although 32 boys were randomly assigned to the PTX group, 2 (6%) withdrew from the study before receiving the first dose; thus, only 30 boys received PTX. Another 32 boys were randomly assigned to the placebo group, and they all began treatment (figure 1). Recruitment took place from November 2005 to December 2006. Participants attended clinic visits at the time of randomization and at protocol-specified intervals for 1 year. Baseline characteristics of study participants are summarized in table 1. The mean age of the study participants was 9.9 (SD 2.9) years in the PTX group and 10.2 (SD 2.8) years in placebo group. Fifty-six (90%) of the participants were Caucasian, 2 (3%) were Asian, and 4 (7%) were of other ethnic origins. Other than corticosteroids, calcium, vitamin D, and multivitamins, none of the participants were taking regular medications or nutritional supplements.

Baseline QMT, MMT, functional scores, and goniometry values were obtained for all participants. Because of disease severity at baseline, there were missing data from 1 (3%) boy in the placebo group who could not perform the 10 m run/walk test, 4 (13%) boys in the PTX group, and 5 (15%) in the placebo group who could not perform the 4 steps climb test and 7 (23%) boys in the PTX group and 7 (21%) in the placebo group who could not stand from supine without assistance. In addition, 2 (6%) boys each from the PTX and placebo groups were unable to cooperate fully with PFTs and total PedsQL scores could not be calculated from 2 (6%) parents in the placebo group at baseline because of incomplete responses.

At baseline, the average of all TGF-β values (both placebo and PTX combined) was 2,394 pg/mL (range 62–15,060 pg/mL). Serum TNF-α levels in the PTX and placebo groups were both nondetectable at baseline.

Outcomes at 12 months. One (3%) participant was lost to follow-up and another withdrew from the PTX group. Two (6%) participants in the placebo group also withdrew during the study (figure 1). There was no significant difference in the primary outcome measure, total QMT score, between PTX and placebo group at 12 months ($p = 0.14$, 95%

Table 1 Summary of study participants between the 2 study arms at baseline

	PTX		Placebo			
Characteristics	n	Mean (SD)	n	Mean (SD)	95% CI for difference	p Value[a]
Age, y	30	9.9 (2.9)	32	10.2 (2.8)	−0.23 (−1.67, 1.21)	0.75
Height, cm	30	124.9 (9.0)	32	124.5 (9.2)	0.44 (−4.18, 5.06)	0.85
Weight, kg	30	31.0 (11.9)	32	29.4 (10.2)	1.57 (−4.10, 7.24)	0.58
BMI, kg/m^2	30	19.3 (4.8)	32	18.6 (4.4)	0.69 (−1.64, 3.01)	0.56
QMT total score, pounds	29	10.6 (4.3)	31	10.8 (3.4)	−0.21 (−2.23, 1.81)	0.84
QMT arm score, pounds	30	7.1 (3.3)	32	7.4 (3.1)	−0.22 (−1.85, 1.41)	0.79
QMT leg score, pounds	30	11.2 (4.8)	32	11.5 (4.3)	−0.31 (−2.63, 2.01)	0.79
QMT grip score, pounds	29	15.7 (7.2)	31	16.3 (5.0)	−0.63 (−3.86, 2.61)	0.70
QMT elbow flexor, pounds	30	7.8 (3.3)	32	8.0 (3.5)	−0.24 (−1.96, 1.48)	0.78
QMT elbow extensor, pounds	30	6.5 (3.5)	32	6.7 (3.0)	−0.20 (−1.87, 1.47)	0.81
QMT knee flexor, pounds	30	11.4 (4.0)	32	12.0 (4.1)	−0.59 (−2.65, 1.47)	0.57
QMT knee extensor, pounds	30	11.0 (6.8)	32	11.0 (5.7)	−0.02 (−3.20, 3.17)	0.99
Total MMT score	30	226.8 (26.6)	32	224.0 (33.1)	2.80 (−12.41, 18.01)	0.71
Walk 10 m, m/s	30	1.46 (0.69)	31	1.67 (0.59)	−0.22 (−0.55, 0.11)	0.19
Climb 4 stairs, 4 stairs/s	26	0.23 (0.14)	27	0.27 (0.13)	−0.04 (−0.12, 0.03)	0.25
Stand from supine, stands/s	23	0.18 (0.10)	25	0.20 (0.10)	−0.02 (−0.08, 0.03)	0.42
FT upper extremity	30	1.2 (0.4)	32	1.2 (0.5)	−0.04 (−0.27, 0.19)	0.75
FT lower extremity	30	2.1 (1.3)	32	2.0 (1.4)	0.08 (−0.59, 0.75)	0.81
FVC, % predicted	28	82.6 (23.2)	28	94.6 (23.5)	−11.98 (−24.50, 0.54)	0.06
FEV1, % predicted	28	84.3 (23.2)	28	94.4 (22.5)	−10.11 (−22.34, 2.13)	0.10
PEFR, % predicted	28	75.2 (20.8)	28	83.4 (19.8)	−8.21 (−19.10, 2.68)	0.14
MIP, cm H_2O	29	48.4 (14.7)	32	45.4 (16.6)	2.97 (−5.06, 11.00)	0.46
MEP, cm H_2O	30	45 (16)	32	44 (15)	0.70 (−7.16, 8.56)	0.86
PF cough, L/min	29	168.8 (50.1)	32	162.7 (49.8)	6.14 (−19.50, 31.78)	0.63
Wrist extension, degrees	30	73.7 (21.6)	32	79.2 (18.2)	−5.55 (−15.75, 4.65)	0.28
Elbow extension, degrees	30	0.7 (6.7)	32	3.3 (6.4)	−2.61 (−5.95, 0.72)	0.12
Knee extension, degrees	30	−0.4 (5.9)	32	0.4 (5.0)	−0.81 (−3.60, 1.99)	0.57
Ankle dorsiflexion, degrees	30	−0.5 (13.0)	32	−1.6 (9.0)	1.14 (−4.59, 6.87)	0.69
Pediatric QOL total score	30	58.5 (15.2)	30	57.1 (15.7)	1.38 (−6.62, 9.38)	0.73
Pediatric QOL physical score	30	50.1 (21.1)	30	49.9 (21.0)	0.21 (−10.68, 11.10)	0.97
Pediatric QOL emotional score	30	65.9 (14.7)	30	65.3 (15.5)	0.58 (−7.24, 8.41)	0.88
Pediatric QOL social score	30	60.9 (18.5)	30	55.3 (19.7)	5.58 (−4.31, 15.48)	0.26
Pediatric QOL school score	30	62.1 (19.1)	30	62.3 (14.3)	−0.17 (−8.91, 8.58)	0.97

Abbreviations: BMI = body mass index; FEV1 = force expiratory volume in 1 minute; FT = functional test; FVC = force vital capacity; MEP = maximum expiratory pressure; MIP = maximum inspiratory pressure; MMT = manual muscle testing; PEFR = peak expiratory flow rate; PTX = pentoxifylline; QMT = quantitative muscle testing; QOL = quality of life; PF cough = peak cough flow.
[a] p Values are based on a two-sided Student t test.

confidence interval 0.63 [0.21, 1.48]) (table 2). Similarly, a trend analysis obtained from the linear mixed-effects model showed no significant difference in the fitted mean of the total QMT scores between the study arms (figure 2). The secondary outcomes also failed to detect any significant differences between the 2 groups for the mean QMT subgroup scores, MMT, functional grading, PFTs, degree of contractures, timed function test, and PedsQL scores except for the timed 10-m run/walk test. The PTX group showed significantly less decline in the velocity to perform the 10-m timed run/walk test after 12 months of treatment than placebo (−0.1 m/second vs −0.3 m/second, respectively; $p = 0.03$, 95% confidence interval 0.16 [0.01, 0.31]). There was no difference between treatment groups in longitudinal trends for total QMT score (figure 2) or any secondary outcomes (data not shown).

Table 2 Comparison between study arms for changes from baseline to month 12

Characteristics	PTX n	PTX Mean (SD)	Placebo n	Placebo Mean (SD)	95% CI for difference	*p* Value[a]
QMT total score, pounds	29	−0.8 (1.9)	30	−1.4 (1.3)	0.63 (−0.21, 1.48)	0.14
QMT arm score, pounds	30	−0.6 (1.4)	32	−1.1 (1.6)	0.45 (−0.32, 1.22)	0.24
QMT leg score, pounds	30	−1.5 (2.9)	32	−1.6 (1.7)	0.11 (−1.12, 1.35)	0.86
QMT grip score, pounds	29	0.1 (3.0)	30	−1.4 (2.9)	1.50 (−0.02, 3.03)	0.05
QMT elbow flexor, pounds	30	−0.5 (1.6)	32	−1.2 (1.7)	0.70 (−0.15, 1.54)	0.10
QMT elbow extensor, pounds	30	−0.7 (1.4)	32	−0.9 (1.8)	0.27 (−0.54, 1.07)	0.51
QMT knee flexor, pounds	30	−1.4 (2.8)	32	−1.3 (1.7)	−0.17 (−1.37, 1.03)	0.78
QMT knee extensor, pounds	30	−1.6 (4.1)	32	−2.1 (2.4)	0.51 (−1.24, 2.25)	0.56
Total MMT score	26	−11.1 (20.1)	30	−15.0 (23.3)	3.88 (−7.74, 15.50)	0.51
Walk 10 m, m/s	30	−0.13 (0.30)	31	−0.29 (0.27)	0.16 (0.01, 0.31)	0.03
Climb 4 stairs, 4 stairs/s	26	−0.05 (0.07)	27	−0.05 (0.06)	−0.001 (−0.04, 0.03)	0.94
Stand from supine, stands/s	23	−0.06 (0.05)	25	−0.05 (0.03)	−0.01 (−0.03, 0.02)	0.51
FT upper extremity	27	0.1 (0.5)	30	0.1 (0.3)	0.06 (−0.17, 0.30)	0.59
FT lower extremity	27	0.4 (0.8)	30	0.3 (1.0)	0.04 (−0.46, 0.53)	0.89
FVC, % predicted	25	−2.5 (14.8)	26	−6.7 (9.8)	4.19 (−2.94, 11.33)	0.24
FEV1, % predicted	25	−2.4 (17.1)	26	−7.8 (11.4)	5.39 (−2.84, 13.62)	0.19
PEFR, % predicted	25	2.4 (22.2)	26	−3.0 (16.4)	5.46 (−5.59, 16.51)	0.33
MIP, cm H_2O	27	4.4 (14.3)	30	3.1 (12.9)	1.36 (−5.89, 8.62)	0.71
MEP, cm H_2O	28	5.5 (11.8)	30	2.5 (13.6)	3.04 (−3.65, 9.72)	0.37
PF cough, L/min	27	−2.0 (47.3)	30	6.1 (33.9)	−8.12 (−30.27, 14.03)	0.46
Wrist extension, degrees	28	2.9 (11.9)	30	2.3 (8.1)	0.61 (−4.80, 6.02)	0.82
Elbow extension, degrees	28	0.5 (5.4)	30	−0.3 (5.4)	0.79 (−2.06, 3.63)	0.58
Knee extension, degrees	28	−1.8 (5.8)	30	0.8 (4.7)	−2.62 (−5.44, 0.20)	0.07
Ankle dorsiflexion, degrees	28	−4.1 (14.8)	30	−1.3 (5.8)	−2.77 (−8.84, 3.29)	0.36
Pediatric QOL total score	28	−1.7 (14.1)	28	3.0 (13.5)	−4.68 (−12.08, 2.71)	0.21
Pediatric QOL physical score	28	−3.0 (20.3)	28	5.2 (23.7)	−8.22 (−20.05, 3.61)	0.17
Pediatric QOL emotional score	28	−2.8 (18.3)	28	2.5 (17.5)	−5.27 (−14.87, 4.34)	0.28
Pediatric QOL social score	28	−1.5 (21.6)	28	0.7 (18.0)	−2.23 (−12.89, 8.42)	0.68
Pediatric QOL school score	26	3.9 (17.0)	28	1.9 (15.3)	2.07 (−6.81, 10.94)	0.64

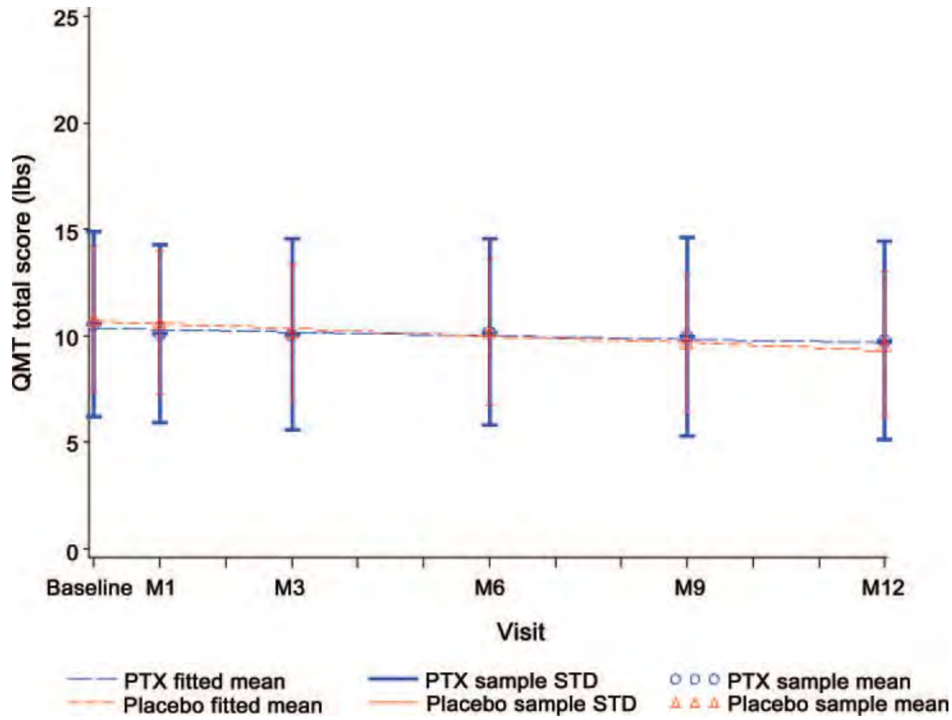
Change from baseline to month 12 (PTX and Placebo columns).

Abbreviations: FEV1 = force expiratory volume in 1 minute; FT = functional test; FVC = force vital capacity; MEP = maximum expiratory pressure; MIP = maximum inspiratory pressure; MMT = manual muscle testing; PEFR = peak expiratory flow rate; PTX = pentoxifylline; QMT = quantitative muscle testing; QOL = quality of life; PF cough = peak cough flow.
[a] *p* Values are based on a two-sided Student *t* test.

AEs. AEs by study arms are summarized in table 3. There were no withdrawals due to medication nonadherence or dose reductions. Mild to moderate AEs were significantly higher in the PTX group as seen by the difference in the proportion of boys with reported gastrointestinal (13 of 30 vs 5 of 32, p = 0.02) or hemorrhage/bleeding (8 of 30 vs 1 of 32, p = 0.01) AEs. There were no significant differences in EKG, PFA, or other safety laboratory measures between the 2 study arms.

DISCUSSION Our study found that the addition of PTX to corticosteroid-treated ambulant boys with DMD failed to slow the decline in overall muscle strength and function after a 12-month treatment period. A number of factors may have contributed to the lack of effect of PTX in this study, despite the positive preclinical results in the *mdx* mouse. First, although the mechanism of action of PTX is through modulation of the TGF-β and TNF-α pathways, the TNF-α pathway was already significantly reduced in corticosteroid-treated boys with DMD, as shown by nondetectable baseline serum TNF-α levels among our study participants. In addition, their serum TGF-β levels were very variable at baseline, which differed from previous studies showing a significant

Figure 2 Trend analysis of quantitative muscle testing (QMT) total score



The fitted mean is the prediction obtained from the linear mixed model. Observations were displayed with sample mean and SD. PTX = pentoxifylline; STD = standard.

increase in plasma TGF-β with DMD.[14,32] It is possible that the increases in the tissue levels of TGF-β and TNF-α may not be reflected in serum, and chronic high-dose corticosteroid treatment may have inhibitory effects on both TGF-β and TNF-α pathways. In addition, our cohort was in the mid to late ambulatory stage, wherein some of the potential effects of PTX on early pathologic events in DMD might be lost.

Preclinical studies in the *mdx* mouse used much higher doses of PTX ranging from 50 to 100 mg/kg/day, which may have played a role in producing the beneficial results.[7–9] In nonhuman primates, doses of 120 mg/kg, but not lower, were effective in attenuating increases of TNF-α, interferon-γ, and interleukin-2.[33] Given the paucity of published data on the safety and efficacy of PTX in children, we were limited to using the maximum safe dosing used in previous studies of 20 mg/kg/day.[20] Under strict monitoring, higher doses of PTX could be considered in future DMD studies. These should also include studies to assess dose-response relationships.

Twelve-month treatment with PTX might not be a sufficient period of time to allow detection of meaningful changes in muscle strength and function. Even though there was no significant difference in the mean total QMT scores after 1 year, it was intriguing to see less deterioration in the 10-m walk/run test ($p = 0.03$) and the QMT grip scores with PTX vs placebo ($p = 0.05$). In addition, although not reaching statistical significance, the decline in most measures was less in the PTX group, a clear directionality that is unlikely to occur by chance. It would be of interest to know whether prolonged or continual treatment with PTX may lead to a more noticeable slowing in the decline of walking speed and muscle strength in selected muscle groups among boys with DMD.

Decreasing fibrosis is a novel approach in DMD, and further studies are needed to understand the appropriateness of pharmacologic modulation of inflammatory pathways using PTX or other antifibrotic treatment in this context. Because PTX was associated with an increase in the risk of mild to moderate gastrointestinal or hemorrhage/bleeding AE in the absence of significant clinical improvement, the addition of PTX to a stable dose regimen of corticosteroids in mid to late ambulant boys with DMD cannot be recommended based on the current study.

Study strengths and limitations. This study tested for the first time an antifibrotic drug as a therapeutic approach for DMD. The study was limited by the paucity of pediatric data on a well-tolerated drug formulation that would allow for more accurate dosing

Table 3 Summary of reported adverse events by study arm[a]

	NCI grade									
	PTX				Placebo					
NCI category	1	2	3	Total	1	2	3	4	Total	p Value[b]
Allergy/immunology	2	1	0	3	2	0	0	0	2	0.67
Cardiac general	0	1	0	1						
Coagulation	0	0	2 (1)	2 (1)						
Constitutional symptoms	12 (9)	7 (6)	0	19 (14)	7 (5)	6 (3)	0	0	13 (7)	0.06
Dermatology/skin	14 (10)	7 (3)	0	21 (12)	8 (7)	2	0	0	10 (8)	0.21
Endocrine					0	1	0	0	1	
Gastrointestinal	21 (12)	2	0	23 (13)	5	1	0	0	6 (5)	0.02
Hemorrhage/bleeding	10 (8)	0	0	10 (8)	1	0	0	0	1	0.01
Infection	22 (13)	7 (6)	0	29 (17)	24 (13)	4	0	0	28 (16)	0.60
Metabolic/laboratory	0	2	1	3 (2)	1	0	0	0	1	0.61
Musculoskeletal/soft tissue	6	5 (4)	3 (2)	14 (11)	3	7	1	1	12 (9)	0.47
Neurology	1	4	0	5	2	4	1	0	7	0.75
Ocular/visual	1	0	0	1	1	1	0	0	2	1
Pain	34 (16)	10 (7)	1	45 (19)	25 (13)	7	0	0	32 (17)	0.42
Pulmonary/upper respiratory	17 (11)	6 (5)	0	23 (14)	11 (9)	5	0	0	16 (11)	0.32
Renal/genitourinary	1	1	0	2						
Surgery/intraoperative injury	0	0	2	2						
Total	141	53	9	203 (29)	90	38	2	1	131 (28)	

Abbreviations: NCI = National Cancer Institute; PTX = pentoxifylline.
[a] Data are presented as n (m) indicating a total of n adverse events from a total of m individuals. NCI Common Terminology Criteria for Adverse Events (CTCAE) grading criteria are shown.
[b] p Values are based on Fisher exact tests.

per body mass and the lack of pharmacokinetics studies that would inform the exposure/benefit relationship. In general, testing of FDA-approved compounds to accelerate the discovery of treatments for such a devastating disease is also limited by the inability to use medicinal chemistry approaches to increase specificity and potency of the drug.

AUTHOR CONTRIBUTIONS

Dr. Escolar designed the clinical trial, conducted the trial as a site principal investigator (PI) and the study PI, participating in analysis and interpretation of data and writing and editing of the manuscript. She revised and approved the manuscript. A. Zimmerman participated in the protocol design, data interpretation, and manuscript drafting. She was the study's Project Manager and site study coordinator. Dr. Bertorini was a site PI. He revised and approved the manuscript. Dr. Clemens was a site PI. She participated in data interpretation and revised and edited the manuscript. Dr. Connolly was a site PI. She revised and approved the manuscript. Dr. Mesa was a site PI. She revised and approved the manuscript. Dr. Gorni was a site PI. She revised and approved the manuscript. Dr. Kornberg was a site PI. He revised and approved the manuscript. Dr. Kolski was a site PI. She revised and approved the manuscript. Dr. Kuntz was a site PI. She revised and approved the manuscript. Dr. Nevo was a site PI. He revised and approved the manuscript. Dr. Tesi-Rocha participated in protocol design and implementation. Dr. Nagaraju participated in analysis and interpretation of exploratory study outcomes. Dr. Rayavarapu participated in analysis and interpretation of exploratory study outcomes. L.P. Hache was the site study coordinator, participated in manuscript preparation and submission and was the study's backup Project Manager. J.E. Mayhew was a study outcome trainer. She participated in analysis and interpretation of data. J. Florence was a study outcome trainer. She participated in analysis and interpretation of data. F. Hu participated in study's statistical design, data analysis, and manuscript preparation. A. Arrieta participated in protocol design and implementation. E. Henricson participated in the design of the protocol, data interpretation, and revision of the manuscript. Dr. Leshner participated in data analysis and interpretation and reviewed the manuscript. Dr. Mah was a site PI and participated in data analysis and interpretation and manuscript preparation. She revised and approved the manuscript.

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DISCLOSURE

Dr. Escolar serves on a scientific advisory board for the NIH/NINDS; serves on the speakers' bureau for and has received funding for travel and speaker honoraria from Athena Diagnostics, Inc.; serves as a consultant for Acceleron Pharma, HALO therapeutics, AVI Biopharma, Gerson Lheman Group (GLC), and Medacorp; and has received research support from the NIH, the Muscular Dystrophy Association, and the Foundation to Eradicate Duchenne (FED). A. Zimmerman serves as a consultant for Halo Therapeutics. Dr. Bertorini serves on the speakers' bureaus of Teva, Biogen Idec, Allergan, and Merck Serono and serves on scientific advisory boards for and receives speaker honoraria from Pfeiffer, Allergan, and Athena. Dr. Clemens receives research support from Genzyme Corporation, Amicus, NIH, Veterans Administration, and Department of Defense. Dr. Connolly receives research support from PTC Pharmaceutical, the Muscular Dystrophy Association and NIH and serves on scientific advisory boards for Acceleron and Halo Therapeutics. Dr. Mesa and Dr. Gorni report no disclosures. Dr. Kornberg has received funding for travel from Genzyme and Biogen Idec and serves as a Section Editor for *BMC Neurology*. Dr. Kolski receives research support from Talecris Biotherapeutics. Dr. Kuntz receives research support from Cooperative International Neuromuscular Research Group/DOD and NIH. Dr. Nevo is listed as an author on a patent re: The use of Glatiramer Acetate in muscular dystrophy and receives research support from AFM and Israeli Ministry of Health and Little Steps (Israeli Parents organization). Dr. Tesi-Rocha reports no disclosures. Dr. Nagaraju serves as an Associate Editor for the *Journal of Neurological Sciences* and is founder of Reveragen Biophrama. Dr. Rayavarapu reports no disclosures. L.P. Hache has received funding for travel and speaker honoraria from Genzyme and receives staff grant funding from NIH and Department of Defense. J.E. Mayhew has received funding for travel and speaker honoraria from Genzyme and serves as a consultant for Enobia Pharma, Inc. and Genzyme. J. Florence has served on scientific advisory boards and/or as a consultant for Prosensa, GlaxoSmithKline, Acceleron, PTC Therapeutics, and DART Therapeutics. F. Hu receives research support from US Department of Defense, NIH, US Department of Education, and the Muscular Dystrophy Association. A. Arrieta receives research support from the US Department of Defense and the Muscular Dystrophy Association. E. Henricson served as a consultant for Genzyme and PTC Therapeutics. Dr. Leshner serves on a scientific board and speakers' bureau for Genzyme and receives research support from Genzyme, Wyeth, and US Department of Defense. Dr. Mah receives research support from PTC Therapeutics, Acceleron Pharma, US Department of Defense, and US FSH Society and Muscular Dystrophy Canada.

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